IDENTIFYING NEWLY SPAWNED FEMALE SARDINES BY MEANS OF COMMON FISHERIES DATA

by

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ABSTRACT. - In the present study we provide an alternative to histology means of discerning newly spawned female sardines, i.e., females with intact postovulatory follicles, by means of common fisheries data like sample sex ratio, gonosomatic index, total length, and fat index. Compared to the remaining yolked females, newly spawned females were shown to occur in samples with increased male-ratios, to have lower gonosomatic index, larger body-size and limited visceral fat reserves. Using these variables each yolked female could be classified correctly as newly spawned with an accuracy of 81%.

RÉSUMÉ. - Identification des sardines femelles qui viennent de se reproduire au moyen de critères de pêche communément utilisés.

L'étude présente propose une méthode alternative à l'examen histologique pour discerner les femelles qui viennent de se reproduire et qui ont donc des follicules postovulatoires intacts. Cette méthode est basée sur des critères de pêche communément utilisés tels que le ratio mâle-femelle, le rapport gonado-somatique, la longueur totale et l'index d'adiposité. Par rapport aux femelles matures, les femelles qui viennent de se reproduire proviennent d'échantillonnages où le ratio de mâles est élevé, ont un rapport gonado-somatique plus faible et des réserves adipeuses viscérales réduites. Cette méthode permet de discerner les femelles qui viennent de se reproduire avec une précision de 81%.

Key words. - Clupeidae - *Sardina pilchardus* - MED - Spawning - Postovulatory follicles - Discriminant analysis.

The assessment of females' spawning condition and the identification of newly spawned females, i.e., females caught immediately or a few hours after the spawning act, are of great importance in the study of the reproductive biology of fish populations. The hourly distribution of newly spawned fish may be used to assess the diurnal spawning activity of a population (Picquelle and Stauffer, 1985; Motos, 1996; Ganias *et al*., 2003). The identification of such fish may also help to determine the exact location of spawning aggregations (Alheit, 1993). Furthermore, the fractions of these females may serve as estimators of spawning frequency, and be consequently used in biomass estimations; e.g., the Daily Egg Production Method (DEPM) (Hunter and Macewicz, 1985).

Despite of its importance, the identification of newly spawned females and of females with a recent spawning act in general, has been hitherto restrained by the laborious and costly histological assessment of postovulatory follicles (POFs). The latter are the only reliable indication of previous spawning activity and may only be detected in histological sections of ovarian tissue (Hunter and Macewicz, 1985). In the present study we provide an alternative to histology means of discerning newly spawned female sardines (*Sardina pilchardus*), by using common fisheries data, like the gonosomatic index, total length, fat index, and sample sex ratio.

MATERIALS AND METHODS

149 samples of adult sardines were collected from coastal locations of the central Greece (Ionian and Aegean) during September 1999 - March 2001 (Fig. 1, Tab. I). Sampling was carried out on board the commercial fleet, as well as the research vessel "PHILIA", by means of a small pelagic trawl. Each sample consisted of a random collection of 1.5-2.0 kg of sardines. Fish were fixed onboard in 10% neutral buffered formalin.

Sex ratio, i.e., the fraction of females by numbers, was determined from a subsample of 50 specimens per catch. Fifteen to twenty females were randomly selected from each catch and measured for total length (L, 0.1 cm) and gutted weight (W, 0.1 g). The amount of visceral fat was subjectively estimated according to a three-point scale: stage-1 = < 25% fat; stage-2 = ≥ 25% and < 50% fat; and stage-3 = ≥ 50% fat. Ovaries were macroscopically scored for maturity

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Table I. - Number (n) of female sardines subjected to histological analysis of their ovaries, fractions of yolked females defined either by histological analysis (H) or macroscopic examination (M), and fractions of yolked females with intact POFs (newly spawned females).

Year	Month	n	H	M	Fraction of newly spawned females
1999	Sep	129	0.00	0.00	0.00
	Oct	179	0.12	0.12	0.00
	Nov	232	0.87	0.86	0.07
	Dec	385	0.75	0.75	0.05
2000	Jan	87	0.93	0.93	0.05
	Feb	156	0.81	0.83	0.07
	Mar	181	0.90	0.91	0.13
	Apr	104	0.72	0.69	0.03
	May	12	0.25	0.33	0.00
	Jun	25	0.64	0.64	0.00
	Nov	87	0.90	0.90	0.10
	Dec	359	0.98	0.98	0.11
2001	Jan	163	0.96	0.96	0.04
	Feb	278	0.97	0.97	0.05
	Mar	15	1.00	1.00	0.00

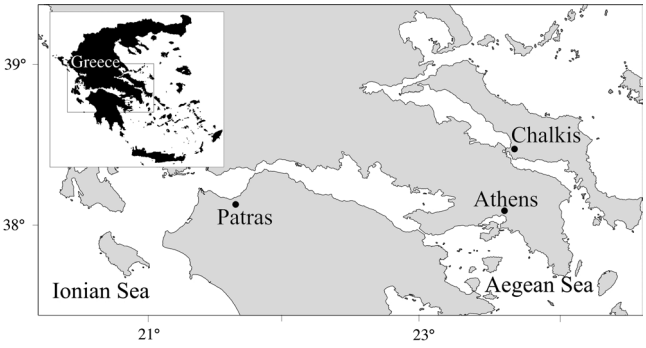

Figure 1. - Map of the study area and main sampling ports.

(yolked or unyolked), dried of surface moisture and weighted (W_0, 0.0001 g). Gonosomatic index (GSI) was calculated as the ratio of gonad weight to gutted weight (W), expressed as a percentage ($GSI = W_o / W*100$).

A piece of tissue was removed from the center of each ovary and subjected to histological analysis (Ganias *et al*., 2003). A total of 2392 ovaries were analyzed histologically. Histological scoring included the maturation stage of the advanced group of oocytes, and the presence and histological characteristics of postovulatory follicles (Ganias *et al*., 2003). In our collections, the postovulatory follicles remained intact, i.e., without any sign of degeneration, only until a few hours after spawning (Ganias *et al*., 2003). Therefore, in the present study newly spawned females were considered to be females containing intact POFs in their ovaries (early POF-0; Ganias *et al*., 2003).

Newly spawned females were examined for differences with the remaining yolked females with respect to four biological traits: sample sex ratio, GSI, total length, and fat-index. The first three variables were compared between the two groups of females using the non-parametric analogue of t-test (Mann-Whitney test; Zar, 1999). Parametric tests were not used because data did not meet the required assumptions. Fat index is a discrete variable, and thus its effect in the two groups of females was quantified using contingency tables analysis (Zar, 1999). Backward stepwise discriminant analysis, based on the generalized Mahalanobis distance, was used to determine the ability of these variables (GSI, sex-ratio, fish-length, and fat-index) to identify the two groups of females correctly.

RESULTS

The results of the histological classification of sardine ovaries to yolked or unyolked were consistent with their macroscopic classification (97%, Tab. I). Newly spawned females, i.e., females with intact POFs in their ovaries, consisted 8% of total yolked females (Tab. I). The former were shown to differ significantly from the remaining yolked females with respect to all the variables examined, i.e., sample sex ratio, GSI, total length, and fat-index. More specifically, newly spawned females occurred in samples with higher percentage of males, had lower GSI and were larger in size than the remaining yolked females (Tab. II). Also, these females had fewer deposits of visceral fat in relation to the remaining yolked females ($\chi^2 = 4.86$, DF = 2, $p < 0.1$).

Backward stepwise discriminant analysis, determined that the all four variables (i.e., sample sex ratio, GSI, total length, and fat index) were significant predictors of newly spawned females, and that the one discriminating function (Tab. III) was statistically significant (Wilk's $\lambda = 0.826$; $F = 88.71$; $p < 0.01$). Using these variables each female could be classified correctly to the two groups with an accuracy of 81% (Tab. IV).

Table II. - Mean values and standard deviation of sex ratio (fraction of females), gonosomatic index (GSI), and total length for newly spawned females and the remaining yolked females, and Mann-Whitney (U) test statistics for the comparison of variables of each group. Minimum and maximum values in parentheses. * $p < 0.05$; ** $p < 0.001$.

Variable	Mean value		U
	Newly spawned	Remaining	
Sex ratio	0.27 ± 0.11	0.55 ± 0.19	22.24**
	(0.10 - 0.44)	(0.08 - 0.98)	
GSI	2.80 ± 0.75	3.96 ± 1.82	64.50**
	(1.32 - 4.80)	(0.26 - 21.86)	
Total length	13.88 ± 0.95	13.48 ± 1.12	88.89*
(cm)	(11.60 - 17.00)	(9.70 - 17.80)	

Variable	Discriminant function coefficients		Classification function coefficients	
	Standardized	Unstandardized	Newly spawned	Remaining
Fat index	0.179	0.383	4.717	5.389
GSI	0.480	0.228	0.466	0.868
Length	-0.189	-0.141	7.568	7.321
Sex ratio	0.949	5.025	8.000	16.806
Constant		-2.109	-57.693	-60.081

Table III. - Discriminant and classification function coefficients.

Group	Percentage correct	Number of yolked females classified into group	
		Newly spawned	Remaining
Newly spawned females	92%	126	11
Remaining yolked females	80%	344	1368
Total	81%	470	1379

Table IV. - Classification matrix.

DISCUSSION

The Mediterranean sardine (*Sardina pilchardus*) is one of the most important small pelagic species, in terms of biomass, in Hellenic waters, representing with anchovy (*Engraulis encrasicolus*) about 30% of the total Hellenic catch (Stergiou and Laskaratos, 1997). It produces multiple batches of eggs in a protracted spawning period which mainly extends at the colder months of the year (Machias and Tsimenidis, 1995; Ganias *et al*., 2001); it also exhibits indeterminate annual fecundity and group-synchronous pattern of oocyte development (Ganias *et al*., in press b). Compared to other populations of the Mediterranean and the Atlanto-Iberian coasts the Hellenic populations of sardine consist of small-sized individuals; their average weight (18 g: Ganias *et al*., 2003) is at least half than the weight of other populations of *S. pilchardus* (41-80 g: Ganias *et al*., 2003). Concerning the reproductive traits of the Hellenic sardine populations the information is sparse and mostly limited to the population of coastal central Greece; length at 50% of maturity (L_{50}) is 11.8 cm (Ganias *et al*., in press a), relative fecundity ranges from 340-360 eggs/g (Ganias *et al*., in press b), and reproductively active females spawn approximately every 11-12 days (Ganias *et al*., 2003).

Newly spawned female sardines, i.e., females with intact POFs, differed from the remaining yolked females with respect to several biological traits. First, such females occurred in samples with high fractions of males. A common feature in the spawning behavior of most epipelagic schooling fish is the formation of spawning schools where individuals (females and males) synchronize spawning (Alheit, 1993). Spawning schools consist of limited in space and/or ephemeral in time segregations of recent and/or imminent female spawners (Ganias *et al*., 2003), which are attractive to high percentages of males (Alheit, 1984; Picquelle and Stauffer, 1985; Ganias *et al*., 2003). Thus, the co-occurrence of newly spawned female sardines with high fractions of males was attributed to the mating behavior of small pelagic fish.

Newly spawned female sardines have lower gonosomatic index and are larger in size compared to the remaining yolked females. Lower values of GSI were attributed to the reduction of ovarian weight, which follows the spawning act and which is generated by the release of the egg batch (Ganias *et al*., 2001). Differences in body-size were attributed to the fact that larger female sardines exhibit higher spawning rate compared to smaller fish (Ganias *et al*., 2003). Thus, newly spawned females are expected to be larger in relation to the average yolked female of the population.

Newly spawned female sardines were also shown to have lower visceral fat reserves in relation to the remaining yolked females. The former have completed at least one full spawning cycle before being captured. Therefore, such females are expected to have already invested more energetic reserves on reproduction in relation to the remaining yolked females of the study, which might include individuals caught at the beginning of their reproductive period still rich in fat reserves.

The present contribution provides evidence that even if intact POFs are impossible to be detected through macroscopic inspection, newly spawned females, which contain such POFs, may be defined without histological analysis by means of simple and low-cost biological data. The definition of such females is very important when studying the reproductive biology of a fish population. The temporal distribution of newly spawned females may approximate the diurnal spawning activity of the assessed population. The latter might be extended to all day-circle (e.g., the Atlantic mackerel: Priede and Watson, 1993) or limited to a specific daily period (e.g., the Mediterranean sardine: Ganias *et al*., 2003). Furthermore, the non-histological assignment of newly spawned females may be intercalibrated with potential scorings of the early stages of POF deterioration in populations

without validated age-key for postovulatory follicles. This is very important for spawning frequency estimations through the postovulatory follicle method (Hunter and Macewicz, 1985), because POF classes are often confused (e.g., Anonymous, 2000). The identification of newly spawned females may also provide information on the location and the extent of the spawning area of a population. Alheit (1993) speculated that fish like sardines and anchovies form spawning aggregations (which fish enter, spawn, and leave), which represent a fraction of the total distribution area of the population. Therefore, instead of costly and laborious egg surveys, these spawning aggregations may be delimited by the simple and low cost definition of newly spawned females.

Acknowledgments. - We thank Dr C. Papaconstantinou, E. Caragitsou and A. Siapatis from the National Centre for Marine Research in Athens and all the staff from the laboratory of zoology of the University of Patras for their collaboration in the collection of the samples. M. Papadaki and N. Papaconstantinou are also thanked for their help in laboratory analysis. This research was funded by a EU Research Project, DG XIV, Contract No 98/0039. This study represents part of the ph.D dissertation of K. Ganias submitted to the Department of Agriculture, Animal Production and Marine Environment, University of Thessaly, Greece.

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Reçu le 30 juin 2003.
Accepté pour publication le 14 octobre 2003.